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Role of Complement in Liver Diseases

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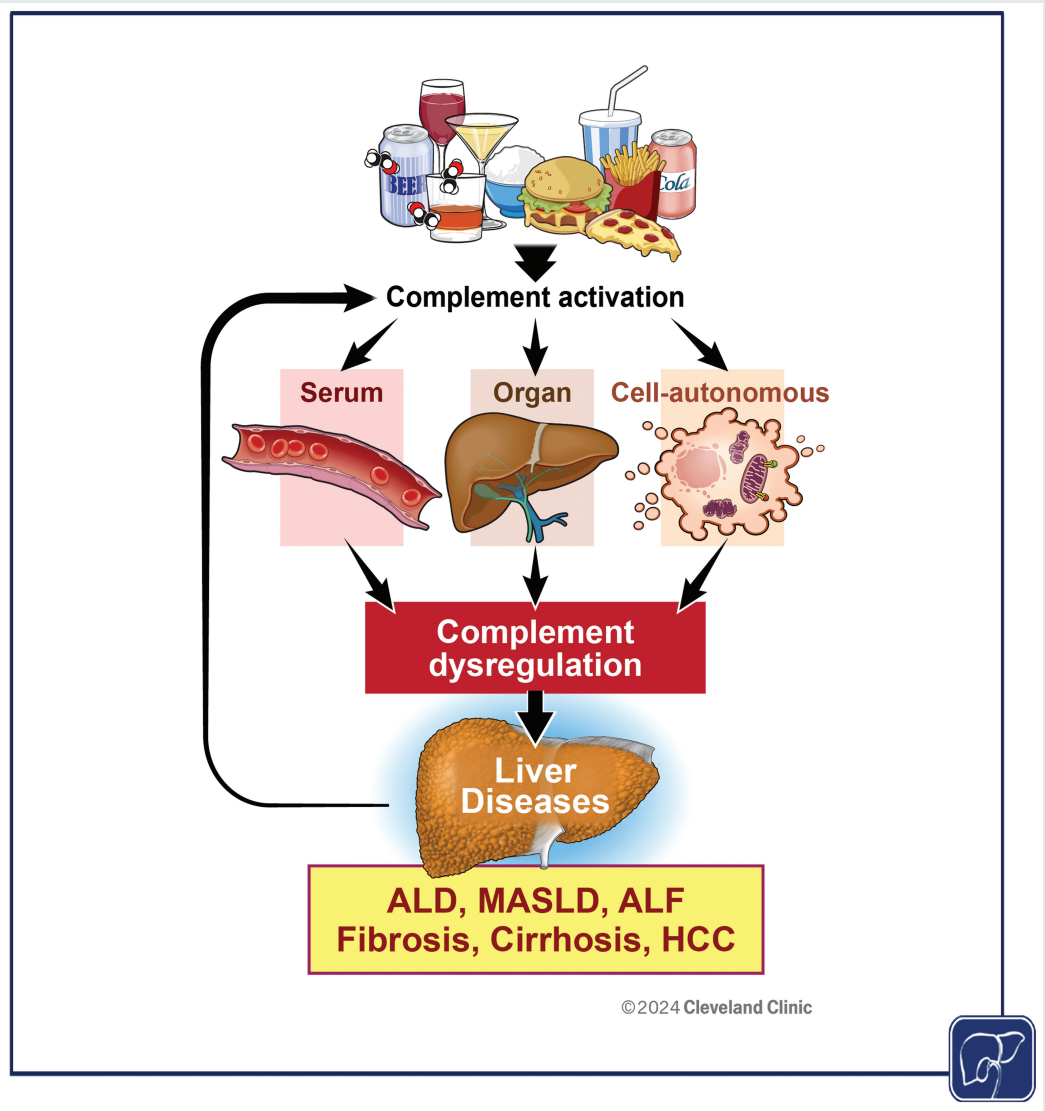
Graphical Abstract



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Abstract

This review aims to summarize recent research using animal models, cell models, and human data regarding the role of complement in liver disease. Complement is part of the innate immune system and was initially characterized for its role in control of pathogens. However, evidence now indicates that complement also plays an important role in the response to cellular injury that is independent of pathogens. The liver is the main organ responsible for producing circulating complement. In response to liver injury, complement is activated and likely plays a dual role, both contributing to and protecting from injury. In uncontrolled complement activation, cell injury and liver inflammation occur, contributing to progression of liver disease. Complement activation is implicated in the pathogenesis of multiple liver diseases, including alcohol-associated liver disease, metabolic dysfunction-associated steatotic liver disease, fibrosis and cirrhosis, hepatocellular carcinoma, and autoimmune hepatitis. However, the mechanisms by which complement is overactivated in liver diseases are still being unraveled.

Keywords

- alcohol-associated liver disease
- metabolic-associated steatotic liver disease
- liver fibrosis
- cirrhosis

Lay Summary

The complement system is composed of soluble and cell membrane-bound proteins that are involved in protecting the body against pathogens and the mediating responses to cellular injury. Although the complement system is highly regulated, it may become overactivated during liver injury and has the potential to cause more liver damage. The specific contributions of complement to the development of liver diseases are still being unraveled.

Complement is part of the innate immune system, composed of over 50 soluble and membrane-bound proteins. The complement system is activated by three different pathways, i.e., classical, alternative, and lectin pathways. All three activation pathways converge at the activation of C3 by C3-convertases. The common terminal pathway culminates with the generation of C5b9 and formation of the membrane attack complex (MAC). Complement activation fragments can also function as opsonins and anaphylatoxins. In recent years, accumulating evidence has revealed an important role for intracellular complement; this system is known as the complosome.[1–4]

When first described, complement was thought to have an exclusive role in the innate immune responses against pathogenic microorganisms. There is a growing appreciation for the importance of both systemic and intracellular complements as integral parts of the innate immune system that go beyond the classically defined role of complement in pathogen resistance. Now, it is understood that complement has critical roles in sterile inflammation, contributing to a pro-inflammatory environment and bridging between innate and adaptive immune systems.[5]

Chronic metabolic liver diseases are associated with increased inflammatory responses and can be characterized as sterile inflammatory diseases.[5–8] Studies from murine models of metabolic liver injury, including alcohol-associated liver disease (ALD) and metabolic-associated steatotic liver disease (MASLD), have revealed an important role of complement in both disease progression and protection from injury.[9–11] Importantly, studies in patients with different types of liver disease, including ALD, MASLD, fibrosis, autoimmune hepatitis (AIH), and hepatocellular carcinoma (HCC), have revealed complex perturbations in complement.[7,12–19]

This review aims to summarize the recent data about complement and its role in the most prevalent liver disease research using animal and cell models and human subjects and is organized in three main sections. First, we present an overview of the complement system and its activation pathways, followed by a discussion of complement-mediated liver diseases, with a description of our understanding for the pathophysiological mechanisms of complement in liver disease and the potential for the development of complement factors as diagnostic and prognostic biomarkers for liver disease.

Complement System

Complement research is dated from the late 18th century, with the report that blood had lower putrefaction compared with other tissues. Since then, complement, called by many other names over time, was characterized, step by step, over the next 200 years.[20,21]

Complement comprises over 50 soluble and membrane-bound proteins.[4,5,22–24] After the 1990s, new technologies and approaches led complement research to another level, revealing the many gaps in our understanding

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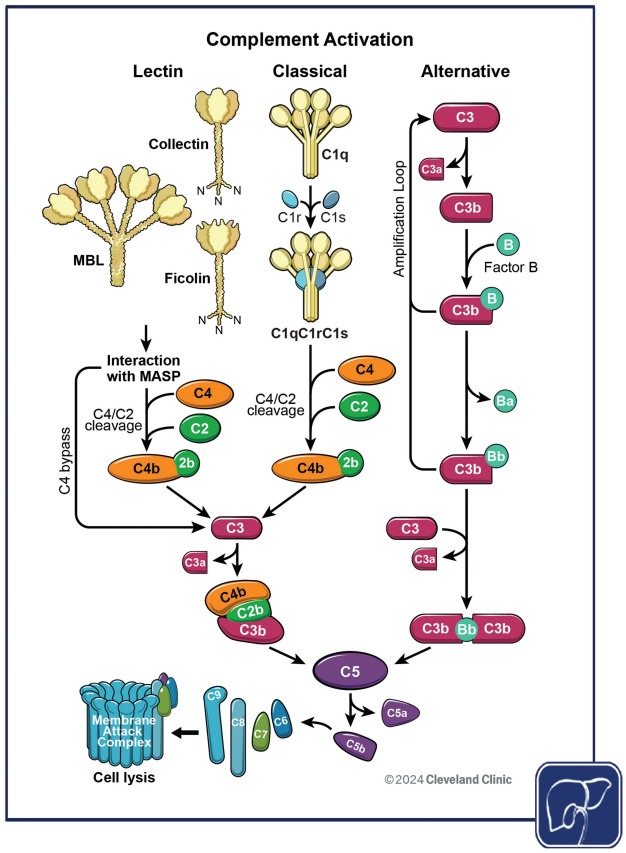

Fig. 1 Complement activation cascade.

of the functions of complement.[21] The liver is the organ that produces the majority of circulating complement components, such as C3 and C4, although other organs and cells are also able to produce complement components, that act mainly in an auto- or paracrine fashion.[25–27]

Complement is activated by three different pathways: classical, lectin, and alternative (►**Fig. 1**).[5,24,28] Classical pathway activation initiates with the binding of C1q to the fragment crystallizable (Fc) region of immunoglobulins and C1r and C1s, serine proteases associated to C1q, cleaves C4 and C2. Cleavage of C4 and C2 generates C4b and C2a and this complex, C4bC2a, also known as C3-convertase, cleaves C3 into C3a and C3b. C4bC2a3b, the C5-convertase, then cleaves C5 and initiates the terminal pathway. The lectin pathway has the same stepwise process as the classical pathway, differing only in how it is activated. Lectins, such as mannose-binding lectin (MBL), ficolins, and other collectins, bind to pathogen-/damage-associated molecular patterns. Association with the MBL-associated serine proteases (MASPs) 1, 2, or 3 makes cleavage of C4 and C2 possible and leads to the same outcome as the classical pathway.[12,24,28–31] The presence of three C-type carbohydrate-recognizing domains is characteristic of collectins, whereas the presence of a fibrinogen-like domain is only associated with the ficolins.[1,4,22,25,28,32–37] Box 1 summarizes the most important points of complement and complement activation pathways.

Box 1 – COMPLEMENT: ACTIVATION AND FUNCTIONS

- The tick-over phenomenon is known as the spontaneous cleavage of C3, generating C3b and C3a. The presence of serum factor H and its binding to the cell membrane are needed for the control of the activation, avoiding deleterious activation.
- The lectin and classical pathways share common components during their activation, although their triggers for activation are different. The lectin pathway needs the association of lectins (i.e., collectins, mannose binding lectin [MBL], surfactant proteins A and D) with MBL-associated serine protease (MASP) 1–3 to cleave C4 and C2. In classical pathway activation, C1q association with the proteases C1s and C1r after the binding to the Fc region of immunoglobulins is the trigger.
- All three pathways converge to the common terminal pathway that leads to C5 cleavage, generating C5a and C5b fragments. The C5b fragment interacts with C6, 7, 8, and 9 to form the membrane attack complex (MAC), which is responsible for pathogen and osmotic cell lysis.
- Complement components have other functions not related to the lysis of pathogens and cell death. For example, C3b can deposit on the cell or pathogen outer membrane and act as an opsonin.
- C3a and C5a are known as anaphylatoxins. They are recognized by their receptors in cell membrane, i.e., C3AR and C5AR1 and C5AR2, and exert functions related to inflammation, cell metabolism, and homeostasis.

The alternative pathway constitutively activated, but at very low rates. The relatively labile thioester bond present in the C3 molecule allows it to transiently activate the so-called "tick-over phenomenon." This permits the alternative pathway to constantly "patrol" for pathogens or injury and also enables rapid responses to pathogen or danger.[38] C3 is cleaved into C3b and C3a. C3b associates with factor B, which is cleaved by factor D, and releases its FBa fragment. C3bBb, the alternative pathway C3-convertase, is now able to cleave more C3, in an amplification loop. C3bBb then associates with another C3b to assemble the alternative pathway C5-convertase, C3bBbC3b.[39,40]

The final step of the three activation pathways is cleavage of C5 and generation of C5a and C5b. C5a and C3a are anaphylatoxins with potent proinflammatory and chemoattractant properties, after their binding to their respective receptors, C5aR1 and C5aR2 (also known as C5L2) and C3aR1.[41–44] The C5b fragment is responsible for the initial formation of the MAC. C5b will then combine with C6 and

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Table 1 Complement: regulators and roles

	Regulators	Role	Ref.
Classical Pathway	C1-INH	• Irreversibly inactivates C1 complex and MASP1/2. • Sequesters C3b, prevents FB binding and C3-convertase assembly.	112
	C4BP	• Binds to C4b and prevents C3-convertase assembly.	112
	CD55	• Decay accelerator of C3 and C5 convertases. • Interacts with both C3 and C5 convertases from alternative pathway and classical pathway/lectin pathway.	112,113
Alternative Pathway	FH	• Binds to cell surfaces. • Acts in the plasma and on cell surfaces. • Cofactor of C3b inactivation with complement factor I.	18,114
	FI	• Inactivates C3b	
Lectin Pathway	C4BP	• Binds to C4b and prevents C3-convertase assembly.	112
	M Alternative pathway-1 (MAp44)	• Competes with MASP for the interaction with MBL, preventing C4 cleavage.	112,115

Abbreviations: MASP, MBL associated serine protease; MBL, mannose binding lectin.

attach to the cell membrane. C7 is responsible for inserting the initial MAC into the bilipid bilayer. C8 and C9 initiate the formation of the pore, the final assembly occurs after the binding of multiple C9 molecules.[45] The C5b9 or MAC is responsible for osmotic lysis of pathogens or cells.[35,46]

In addition to the formation of MAC, C3 fragments generated by complement activation also contribute to opsonization of pathogens and damaged cells.[38,40,47–51] In the liver, the CRIg (complement receptor of immunoglobulins superfamily) receptor expressed by Kupffer cells is responsible for clearing opsonized pathogens, cellular debris, and immune complexes. For example, CRIg binds to C3b and iC3b and is necessary for opsonized *Listeria monocytogenes* and *Staphylococcus aureus* clearance from the blood by the liver in mice.[51]

C1q, one of the activators of the classical pathway, also has opsonizing properties. C1q can bind to oxidized low-density lipoprotein (LDL) and enhance its phagocytosis by macrophages.[52,53] The presence of C1q in the oxidized and acetylated LDL reduces the expression of apoptosis-related genes in foamy macrophages. Also, C1q is responsible for the enhancement of phagocytosis and efferocytosis by foamy macrophages.[54]

In humans and nonhuman primates, erythrocyte CR1 plays a significant and important role in the clearance of pathogens and immune complexes. After binding to the epitope, C1q recruits C1r and C1s resulting in the activation of the classical pathway, which in turn generates C4b and C3b, which then bind to the pathogen/immune complex. C1q, C3b, and C4b present on the surface of the pathogen/immune complex, then binds to CR1. Kupffer cells recognize and phagocytose the pathogen/immune/complement complex adhered to the erythrocyte. This form of opsonization and clearance is called immune adherence.[55] Damaged T cells are also phagocytosed by macrophages, in a mechanism that depends on complement-mediated opsonization. In vitro, the use of Cp40, an inhibitor of complement convertases, reduces the phagocytosis of damaged T cells by macrophages. Similarly, heat inactivation of serum also reduces T cell phagocytosis. Recognition of complement C3 fragments deposited on T cell membranes is mediated by CR1, CR3, and CR4; blockage of the three receptors also reduces phagocytosis of T cells.[50]

The three pathways of complement activation are closely regulated by complement regulatory proteins. Regulators are either soluble (i.e., C1-INH, C4BP, FH) or membrane-bound (i.e., CD59, CD55, CR1).[3,23] Assembly of C3-convertase (C4b2a) in response to activation of the classical and lectin pathways is negatively regulated by C4b-binding protein (C4BP). Studies suggest that binding of C4BP to apoptotic and necrotic cells prevents C3b deposition and subsequent complement activation. Negative regulation by C4BP is particularly important for preventing induction of autoimmune and inflammatory responses in response to the release of DNA from necrotic cells.[56,57] Another important complement regulator is complement factor I (CFI). It inhibits all complement pathways primarily by inactivating complexed C3b, as well as C4b. CFI deficiencies are associated with atypical hemolytic uremic syndrome and recurrent infections by encapsulated pathogens.[37,58,59] Patients with alcohol-associated hepatitis (AH) also present with low plasma concentrations of CFI and CFI is negatively correlated with severity of AH.[34]

Complement factor H (CFH), an alternative pathway soluble regulator, has a direct role in preventing overactivation of C3 via the "tick-over phenomenon." In mice lacking CFH, alternative pathway activation, evidenced by C3b and iC3b deposition on the surface of sinusoidal endothelial cells, is constitutively activated and associated with overexpression of proinflammatory cytokines and development of hepatic tumors.[18] The role and action of some complement

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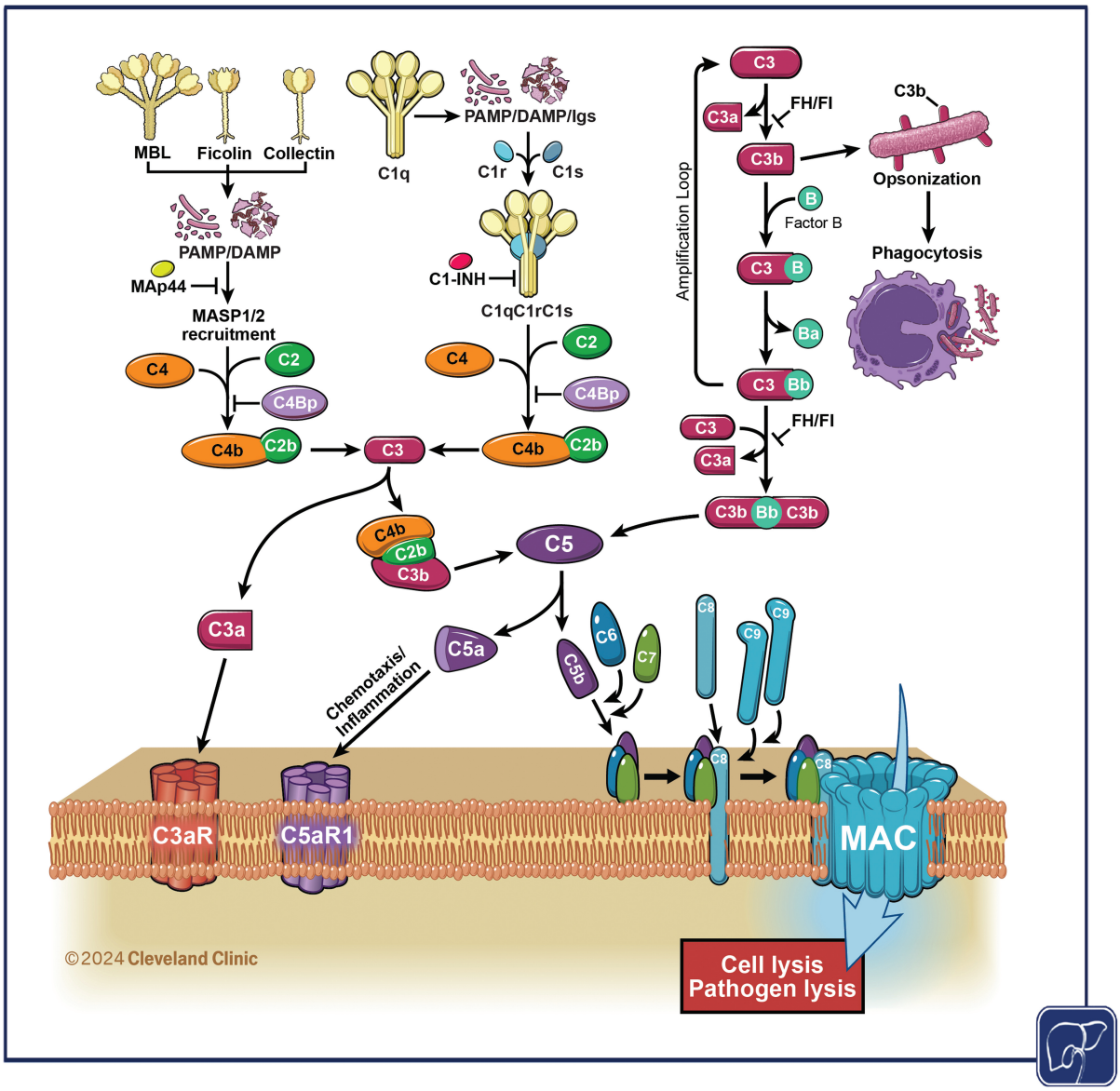

Fig. 2 Schematic of the overall complement cascade.

regulators is depicted in ►**Table 1** and a schematic of the overall complement cascade is shown in ►**Fig. 2**.

Recent research has focused on the novel roles of intracellular complement activity. Intracellular complement, or complosome, plays important roles in cellular homeostasis, such as metabolism, autophagy, and gene expression, as well as in disease.[1–3,42,60–62] The presence of core complement proteins and convertases, regulators. and complement receptors, such as C3, C3a, C3b, C5, C5aR1, and C5aR2, on various intracellular organelles indicates the importance of the noncanonical intracellular complosome.[2,33,42,60–64]

In contrast to activation of serum complement, intracellular complement activation induces responses related to cell metabolism and survival.[2,63] The complosome also contributes to T cell polarization toward Th1, Th2, or Th17.[65] Resting $CD4^+$ T cells depend on intracellular C3 activation by cathepsin L protease for their survival and the activation of main metabolic pathways. Although $CD4^+$ T cells depend on intracellular C3, extracellular C3 cleavage is also necessary to induce interferon-γ production by $CD4^+$ T cells. Interestingly, after activation, C3aR and CD46 migrate from intracellular organelle membranes to the plasma membrane and have an active role in T cell activation and production of proinflammatory cytokines.[63]

Another important feature of intracellular complement activation was described in macrophages. Macrophages can assemble C5-convertase in the cytoplasm and express C5aR1 receptors on the mitochondrial outer membrane. C5aR1 and intracellular C5a are involved in interleukin-1β secretion and contribute to sterile inflammation. One example of this proinflammatory role for the complosome was evidenced in mice fed with a high-fat diet: deletion of macrophage C5aR1 resulted in a 50% reduction in atherosclerotic plaques in mice.[42] These recent data highlight the involvement of

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intracellular complement in the response to inflammatory triggers and point to another noncanonical role of complement.

Complement and Liver Disease

ALD and AH

Multiple studies, by our laboratory[9,11,34,43,47,48,66] and others,[31,67] have demonstrated evidence for complement activation in both murine models of ALD and in patients with AH. The C3 activation product, C3-C3b-C3i, is detected early in the response to ethanol feeding in mice and *C3* or *C5* deficiency protects mice from chronic ethanol-induced liver injury. In contrast, deficiency in the complement regulator, *CD55,* exacerbated injury.[31]

The stimuli for complement activation in response to ethanol feeding are not completely understood. Studies have implicated the association of C1q with apoptotic hepatocytes and activation of the classical pathway. In wild-type mice, but not $Bid^{-/-}$ mice deficient in apoptosis, chronic ethanol induces hepatocytes apoptosis. Associated with increased apoptosis in wild-type, but not $Bid^{-/-}$, mice, secretory immunoglobulin M deposits accumulate in the liver, colocalizing with C1q and C3-C3b-iC3.[66] Taken together, these data suggest that the classical pathway, recognizing injured/apoptotic hepatocytes, is activated, contributing to hepatic inflammatory responses after ethanol feeding.

Additional data suggest that multiple pathways of complement activation may be involved in the response to chronic ethanol feeding. Using mice deficient in *C1q*, *C4*, *FD*, and *C1q/FD,* McCullough et al[47] showed that $FD^{-/-}$ mice, i.e., mice deficient in the alternative pathway, had more severe steatosis, liver inflammation, and oxidative stress compared with wild-type or $C1q^{-/-}$ (classical pathway–deficient), $C4^{-/-}$ (classical/lectin pathway–deficient), and *C1q/FD* double knockout mice. These results demonstrate that the alternative pathway activation can have protective functions during chronic ethanol feeding in mice.

Adding to the complexity of complement activity in response to ethanol, subsequent data suggest that the role of complement in murine models of ethanol-induced liver injury is more complex. For example, while global $C5ar1^{-/-}$ mice have reduced inflammatory responses in the liver, markers of hepatocyte injury, including elevated plasma alanine aminotransferase, hepatic triglycerides, markers of oxidative stress, and apoptosis, are exacerbated compared with wild-type mice. Studies in chimeric mice (WT → $C5aR^{-/-}$ and $C5aR^{-/-}$ → WT) revealed that while *C5ar1* on myeloid cells contributes to inflammatory injury, *C5ar1* expression by nonmyeloid cells protects them from chronic ethanol-induced hepatocyte injury.[43]

Gut bacterial translocation and presence of bacterial components, such as lipopolysaccharide (LPS) and lipoteichoic acid, are also involved in the pathogenesis of ALD by direct action on Kupffer cells and hepatocytes, but also by activation of complement-related pathways. The CRIg is expressed by Kupffer cells and interacts with C3b, iC3b, and C3c deposited on bacteria during opsonization.[51] Patients with AH have less CRIg, associated with fewer Kupffer cells in the liver and inflammation-related downregulation of CRIg.[68] Although the reduction of CRIg may contribute to injury in patients with AH, total ablation of *CRIg* in mice results in more severe liver injury after a chronic plus binge ethanol exposure. This receptor is involved in recognition and clearance of gram-positive bacteria, and counts of these bacteria were higher in the *CRIg* knockout mice. CRIg downregulation, associated with TRL2 recognition of gram-positive bacteria, leads to increased inflammation and liver injury. Knocking out CRIg and TLR2 ($CRIg^{-/-}$, $TLR2^{-/-}$) decreases liver inflammation, due to decreased bacteria recognition, which shows that a decrease in CRIg reduces bacterial clearance and induces liver damage by TLR2-associated inflammation.[68]

In patients with AH, data suggest that all three complement pathways are activated. Analysis of liver biopsies from patients with AH found that accumulation of the active cleavage fragment of FBb, but not FBa, is increased. Further, C3 activation was evidenced by the presence of C3b, iC3b, and C3c in the liver of patients with severe AH, but not in the liver of healthy donors. Analysis of hepatic messenger RNA (mRNA) expression showed increased expression of C1q, C3aR, and C5aR in liver biopsies from individuals with AH.[47]

Recently, our group reported that complement may be of significance as diagnostic and/or prognostic biomarkers in AH. In a correlation analysis, circulating concentrations of C2, C4b, C4d, CFI, C5, and sC5b9 were negatively correlated with severity of AH, while FBa and complement factor D (CFD) were positively correlated. Specific serum complement proteins also discriminate between mild and severe AH. CFI and sC5b9 presented moderate ability to discriminate between mild and severe AH, but CFI had higher sensitivity compared with sC5b9.[34]

Using proteomics data from serum and liver explants of patients with AH, we reported that complement components are differentially expressed in patients with AH compared with healthy controls, heavy drinkers, and patients with alcohol-associated cirrhosis. Collectin 11, a collectin involved in activation of the lectin pathway, and CD59, a complement regulator, can be used as biomarkers to differentiate AH from healthy individuals and heavy drinkers. To confirm the proteomics analysis, measurements of collectin 11 in the serum and CD59 in liver explants, by ELISA (enzyme-linked-immunosorbent assay) and western blot, respectively, were performed. Collectin 11 and CD59 were both increased in AH individuals compared with healthy controls. In addition, collectin 11 and C1q binding protein can differentiate patients with severe AH and alcohol-associated cirrhosis with high sensitivity and specficity.[69]

Metabolic Dysfunction–Associated Steatotic Liver Disease

Recently, the nomenclature to describe the pathological alterations seen in nonalcohol-associated liver disease (NAFLD) was changed to metabolic dysfunction-associated steatotic liver disease (MASLD).[70,71] In the published consensus, all liver diseases with any degree of steatosis are classified under the umbrella of steatotic liver disease (SLD).

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Patients with steatosis and at least one cardiometabolic diagnosis are classified under the term MASLD. Those with metabolic dysfunction and steatohepatitis are classified as MASH (metabolic dysfunction-associated steatohepatitis). Finally, although patients with ALD may experience steatosis, and thus would be expected to be under the SLD umbrella, there are differences between patients with ALD and MASLD. To clarify this, the consensus statement adopted the term MetALD for those patients with MASLD who drink over the limit of 140 to 130 g/week and 210 to 420 g/week for females and males, respectively. Patients with MetALD may experience symptoms and have characteristics of both conditions, and one or other may be predominant.[71]

Obesity and metabolic syndrome are associated with complement dysregulation, thus complement may contribute to the incidence of NAFLD/MASLD.[14,71,72] Adipose tissue produces the adipokines, such as leptin, adiponectin and resistin, and multiple complement components, including adipose-derived factor D/adipsin, CFB, CFH, C3, and acylation-stimulating protein.[72–74] C3 concentrations are elevated in NAFLD cases, and positively correlated with disease severity.[14] In a cross-sectional study with 7,540 participants performing annual checkup in a hospital in China, all of those that were diagnosed with NAFLD had higher levels of C3 compared with patients without NAFLD. Patients with serum C3 levels >1.07 g/L were more prone to have NAFLD than those with lower levels of C3. C3 serum levels were also associated with severity of the disease (ultrasonographic steatohepatitis and abnormal levels of hepatic enzymes). One interesting feature found in the study was that lean and metabolic syndrome-free patients with NAFLD also had higher C3 serum levels. These results showed that C3 levels are an independent risk factor for NAFLD in the studied Chinese population.[13]

Conversely, in mice, intracellular C3 participates in cell lipid metabolism and *C3* knockout (KO) mice have more accumulation and higher number of lipid droplets in hepatocytes, compared with wild-type mice. The circumference of lipid droplets was also larger in the *C3* KO mice compared with wild-type. Intracellular C3 is involved in lipophagy in hepatocytes, thus deficiency of C3 contributes to lipid droplet accumulation and hepatic steatosis.[75]

Feeding high-fat and high glucose diets to mice is a common murine model of metabolic syndrome. Ingestion of high fat plus high glucose diet by C57BL6 $C5^{+/+}$ mice and $C5^{-/-}$ for 10 weeks resulted in more macrovesicular and more aggressive grade steatosis combined with more severe centrolobular inflammation in the wild type, compared with $C5^{-/-}$. The presence of C5 directly regulated the occurrence and grade of steatosis, as well as inflammation.[6] Complement component C4 is positively correlated with the occurrence of NAFLD. Serum C4 levels are higher in patients with NAFLD than those considered healthy.[14]

In the past few years, the concept of "complotype" has emerged. Complotype is the set of individual genetic signatures that influence how the individual will respond to pathogen aggression, but also applies to other roles of complement, such as sterile/metabolic liver disease. Genetic polymorphisms may influence the activation of the alternative pathway, in particular, and dictate the occurrence of diseases related to overactivation or an impaired response capacity of the complement regulator proteins.[76] The $C3_{R102G}$ polymorphism by itself leads to increased alternative pathway activity. In vitro purified $C3_{102R}$ and $C3_{102G}$ from homozygous donors evidenced the higher activity of the $C3_{102G}$ due to less efficient binding of FH, thus leading to alternative pathway overactivation. In a similar experiment, using the three most commonly described alternative pathway polymorphisms, i.e., $C3_{R102G}$, fB_{Q32R}, and fH_{I62V}, alternative pathway activity, measured by hemolysis, was sixfold higher than in the nonmuted proteins.[77]

Liver Fibrosis and Cirrhosis

Liver fibrosis is one of the common pathological features encountered in advanced liver diseases of multiple etiologies and evidence indicates a role for complement activation in the progression of liver fibrosis.[48,78–81] Chronic infection with hepatitis virus B and C (HBV and HCV, respectively) leads to complement activation and fibrosis. Also, the presence of circulating immunoglobulin A (IgA) against HBV may indicate progression of infection and is associated with liver fibrosis in HBV infection.[82] Although the presence of higher levels of IgA is observed in patients with chronic HBV infection, both IgA and C4 are independent risk factors for liver fibrosis.[79]

The anaphylatoxins C5a, C3, and C4 are reduced in plasma of chronic infected HBV patients and there is a marked reduction in C5a, C3, and C4 concentrations due to liver fibrosis. There is a negative correlation between C5a and the severity of liver fibrosis and the early stages of cirrhosis.[81] Reduction in the levels of C3 and C4 was negatively correlated with fibrosis stages, and IgG levels were positively correlated with liver fibrosis stage.[80]

Hepatic stellate cells (HSCs) play a critical role in the development of liver fibrosis. Working with mice primary HSC cells, C5a was found to be a potent chemoattractant for activated HSC migration in a two-dimensional wound healing assay and in the Boyden chamber cell migration assay. C5a was as potent as the well-described chemoattractant, platelet-derived growth factor.[83]

In the murine model of acute liver injury induced by carbon tetrachloride (CCl_4), tissue injury is exacerbated due delayed or lack of removal of cell debris. Since the alternative pathway plays a role in opsonization and removal of damaged cells, absence of the alternative pathway is associated with persistence of dead cells and sustained inflammatory responses.[48] For example, mice deficient in alternative pathway activation, as well as those lacking plasminogen activators, have more severe liver injury compared with their wild-type controls, associated with impaired clearance of cellular debris.[84] Mice that lack CFD have less C3b, iC3b, and C3c deposition in the liver after CCl_4 acute injury. This impaired opsonization activity results in fewer cells targeted for clearance, which increases liver injury and inflammation.[48]

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Hepatocellular Carcinoma

The occurrence of HCC is linked to many risk factors such as tobacco use, HBV and HCV infection, MASLD and diabetes, and ALD.[85] Although there are many risk factors associated with occurrence of HCC in humans, complement activation likely has a role in HCC. Sustained liver inflammation, due to dysregulated complement activation, especially via activation of the alternative pathway, is one of the causes of HCC in mice. Mice that lack FH ($fh^{-/-}$), a soluble regulator of the alternative pathway, have a higher incidence of HCC in older male mice when compared with wild-type and heterozygous ($fh^{+/-}$) mice (56, 7, and 10%, respectively). The activation of C3 in those mice was confirmed by staining C3-C3b-iC3b/C3d in the liver. The deposition of C3-C3b-iC3b/C3d was linear and continuous and was more concentrated close to the central vein. Interestingly, mice that were $fh^{-/-}$ and $fb^{-/-}$ had minimal to no alternative pathway activation or C3b, iC3b/C3d deposition in the liver, since factor B is necessary for the alternative pathway activation and assembly of the C3-convertase C3bBb. In accordance with the findings in mice, human liver biopsies stained for C3d/iC3b and glypican-3 (an HCC marker) presented colocalization of both staining, demonstrating that the alternative pathway is activated in human HCC.[18]

In contrast, CFH is essential for HCC metastasis. Extracellular vesicles (EVs) from HCC metastatic cell lines analyzed by mass spectrometry had higher levels of FH than the nonmetastatic HCC cell lines. One HCC metastatic cell line, MHCC97L, was orthotopically implanted in the liver of mice and, 5 weeks after implantation, EVs containing FH were detected in the circulation. The EV-associated CFH augmented tumor growth in the liver as well as facilitated lung metastasis. It is likely that the complement inhibitory function of CFH prevented the formation of MAC on tumor cells, thus enabling tumor growth and metastasis.[86]

In three different datasets of bulk mRNA expression in HCC, the most down-regulated complement factors were C1R, C6, C7, C9, CFP, and CFHR3. Although these mRNAs were consistently downregulated in the three datasets, other complement-related mRNA varied between the datasets (up- or downregulated). Patients with higher expression of the above listed genes had better survival rates than the ones with lower expression, and patients with more advanced stages of HCC had lower levels of mRNA expression of the genes of CR1, C6, and CFHR3.[87]

One important feature of HCC is the occurrence of epithelial-to-mesenchymal transition (EMT). In patients with clinically and histologically confirmed HCC, both complement receptors C3aR and C5aR1 are overexpressed in the tumors' cells. In vitro studies also showed that downregulation of both C3aR and C5aR in HCC cell lines suppresses cell proliferation and induces cells death by apoptosis, as well as upregulation of E-cadherin and down-regulation of N-cadherin and vimentin.[17] In the same HCC cell line, stimulation with aristolochic acid, a known liver carcinogen, led to C3a and C3aR overexpression accompanied by enhanced invasion ability and overexpression of EMT-related proteins. These alterations are abrogated with C3aR blocking by a C3aR antagonist.[19]

Complement impacts both cancer development and treatment, its roles in these processes are likely dictated by the tumor microenvironment. Blockade of proinflammatory anaphylatoxin receptors, i.e., C5ar1, may improve antitumor PD-1/PD-L1 checkpoint inhibition by monoclonal antibodies (mAbs), in lung and hematopoietic cancers.[88–90] In addition, complement activation by C1q binding to the Fc portion of mAbs leads to MAC formation and tumor cell death.[90,91] In contrast, in HBV-induced HCC, C4BP is upregulated in infected cells, inhibiting formation of the MAC complex providing resistance of the HBV-infected cells to complement-mediated lytic cell death. On the other hand, ficolin-3 expression in HCC cells leads to lipid accumulation and ferroptosis-mediated cell death.[92,93]

Acute Liver Failure

The lectin pathway is activated by the binding of lectins to their targets and its activation leads to C4 and C2 activation and C3-convertase formation. Patients with acute liver failure have significantly lower levels of MBL and M-ficolin and higher levels of collectin-11 compared with healthy controls. Interestingly, patients with higher MBL levels at day 1 had higher spontaneous survival rates than those with lower levels. Collectin-11 was high in patients with ALF, but even higher in patients with cirrhosis. This shows a dynamic change in the lectin pathway proteins during ALF, but also during cirrhosis, a chronic liver disease.[29]

Complement component C5 plays a role in the development and degree of ALF in a mouse model using LPS/D-galactosamine hydrochloride (D-GalN). *C5* KO mice and those treated with a humanized anti-C5 mAb had lower levels of ALT (hepatocyte injury marker) and injury grade compared with wild-type. *C5* KO mice treated with the anti-C5 antibody groups had the highest survival rate, followed by *C5* KO and WT-treated groups, both in short- and long-term survival studies.[94]

Autoimmune Liver Disease

There are three types of autoimmune liver diseases, i.e., AIH, primary biliary cholangitis, and primary sclerosis cholangitis.[7]

AIH is another hepatic disease that involves the immune system reacting against the liver. AIH often leads to more advanced liver diseases, including cirrhosis or liver failure. Diagnosis may be difficult in some cases, but elevated IgG, autoantibodies, and specific histological findings are present in all cases. The involvement of complement in the pathophysiology of AIH has been studied, with the classical and alternative pathways described as important players in the disease.[16,95]

Complement plays critical roles in the development of AIH. Together with higher C3a levels (complement activation) in patients with AIH compared with healthy subjects, lower circulating CFH and MASP-2 are also associated with occurrence and severity of AIH.[16] Deposition of C4d is found in the liver of AIH patients after liver transplantation. C4d deposition was observed in 18.2% of patients with recurrent

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AIH compared with 13.6% of patients with de novo AIH (patients who develop AIH after transplantation with no history of previous AIH).[15] The C4d deposits are considered a good immunohistochemical marker of liver rejection and is found, as mentioned, in the liver of transplant recipients.[96]

Since IgG levels are elevated in the plasma of patients with AIH, its deposition in the liver is also used as a diagnostic tool. Concurrently, detection of C3 and C4 inside the hepatocytes is also seen in those patients. However, caution must be exercised when evaluating C3 and C4 in hepatocytes, as the liver is the main producer of both complement components. C5b9 is used as a marker of complement activation, but in a set of nine patients with AIH, only one presented immunohistochemical evidence of C5b9 deposition.[7]

Prognosis of Liver Diseases

The specific etiology of the liver disease in patients is often difficult to ascertain solely on clinical parameters, often impeding diagnosis and treatment. Due to late diagnosis and, in most cases, disease severity, patients with liver disease have a poor prognosis.[97–103] Therefore, research into the identification of noninvasive biomarkers of liver disease is an area of active investigation, with a goal to improve diagnosis, prognosis, and theragnosis. Since multiple complement factors are perturbed in the circulation of patients with liver disease, many investigators are working to develop complement as liver disease biomarkers.[34]

Expression of complement genes in patients with different liver diseases informs prognosis of the disease. For example, in HCC, downregulation of C1R, C6, C7, CFP, and CFHR3 is associated with poor survival. Also, reduction in the mRNA expression of these proteins is associated with advanced tumor stages.[87] Lower concentrations of CFI and sC5b9 in plasma of individuals with AH were associated with lower 90-day survival.[34]

In liver fibrosis and cirrhosis, the serum concentration of C1q has good prognostic value. Individuals with values under 11 mg/dL C1q were less likely to die, compared with those with values over 11 mg/dL.[104] In individuals infected with HBV, lower complement C3 was correlated with the occurrence of acute on chronic liver failure (ACLF) in patients with decompensated cirrhosis, and plasma C3 concentration can predict the occurrence of ACLF in these patients.[105]

Table 2 Complement-associated liver diseases

Liver disease	Characteristics	Ref.
Alcohol-associated liver disease	• C3aR- and C5aR-mediated TNF-α production. • C1q deposition in conjunction with sIgM. Lack of sIgM is protective. • In patients with AH: increase of active cleavage fragments of alternative and classic pathways, such as CFB and FBb. Increased expression of C1q, C3aR, and C5aR genes. • Reduction of CRIg in humans and mouse. Increased liver damage in *CRIg* KO mouse. • C2, C4b, C4d, CFI, C5, and sC5b9 are negatively correlated with AH. FBa and CFD are positively correlated with AH. • Collectin 11 is increased in the serum of AH individuals, and CD59 is increased in liver tissue of the same individuals.	11 66 47 68 34 69
Metabolic-associated steatotic liver disease	• Increased concentration of serum C3. • C5 is related to steatosis and centrolobular inflammation in mice. • Serum C4 concentration is higher in patients with MASLD (NAFLD).	13,116,117 6 14
Liver fibrosis and cirrhosis	• C3 and C4 concentrations are negatively correlated with fibrosis. • CFD is involved in cell clearance and protection in the carbon tetrachloride model of liver fibrosis.	80 48
Hepatocellular carcinoma	• Alternative pathway dysregulation is associated with the occurrence of HCC in mice. • Factor H is essential for HCC metastases. • C1R, C6, C7, C9, CFP, and CFHR3 are downregulated in patients with HCC.	18 86 87
Acute liver failure	• Lower levels of MBL, M-ficolin, and collectin 11. • C5 is correlated with the development and degree of ALF in mice.	29 94
Autoimmune hepatitis	• Classic and alternative pathways are involved. • High C3a and low CFH and MASP2 concentrations are associated with disease degree.	16,95 16

Abbreviations: AH, alcohol-associated hepatitis; HCC, hepatocellular carcinoma; KO, knockout; MASLD, metabolic dysfunction-associated steatotic liver disease; MASP2, MBL associated serine protease 2; NAFLD, nonalcoholic fatty liver disease; sIgM, secretory IgM; TNF-α, tumor necrosis factor-α.

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The role of complement in the progression of liver lesions is beginning to be understood; however, their utility as biomarkers and diagnostic and prognostic indicators still needs more studies and validation.[106] ►**Table 2** highlights the complement-associated liver diseases discussed throughout this review and gives a brief characteristic of them.

Complement-Directed Therapeutics

Despite the strong basic and clinical scientific evidence for a role of complement in the progression of liver disease, at this time, there are currently no Food and Drug Administration-approved drugs that directly target complement in liver disease.[107] Most of the currently available drugs that target complement are mAbs or small-molecules or peptides. For example, Eculizumab, a mAb that targets C5, is primarily used to treat paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome,[107–109] while additional drugs inhibiting C5, C3, CFD, and CFB are approved or are in phase 3 clinical trials for multiple other inflammatory diseases.[108,110,111] The efficacy of drugs targeting inhibition of complement activation for several inflammatory diseases holds promise for the eventual adaptation of complement therapeutics for treatment of liver disease and its complications.

Concluding Remarks

Since its first description in the 18th century, much progress has been made to unravel the function of complement in health and disease. Liver diseases are a growing concern worldwide, and the participation of complement in their pathogenesis also needs further studies to dissect specific mechanisms for complement activation as well as the complex interactions between the different arms of the complement system.

The role of the cell-autonomous complement system in the occurrence and maintenance of liver injury is still open to important discoveries. Given the role of intracellular complement in other inflammatory diseases, intracellular complement likely participates in the development of liver diseases.

Another important subject that needs to be addressed is the crosstalk between liver and other organs during liver injury, including, but not limited to heart, adipose, muscle, and kidney. Since the liver is the most important organ to produce and secrete most of the soluble complement components, hepatic dysfunction in liver disease is likely to perturb critical organ–organ interactions, further exacerbating the health of the organism.

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Conflict of Interest
None declared.

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